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Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples.

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Abstract:

Background: Glyphosate has recently received much public attention following its 'Group 2A – probably carcinogenic to humans' classification from the International Agency for Research on Cancer. Despite the widespread use of glyphosate, there is limited data on potential exposures during common occupational uses.

Objective: The study aimed to characterise occupational exposures to glyphosate among amenity horticulturists through the collection and analysis of urine samples following pesticide application. The impact of work practices on personal exposure as well as suitability of collecting multiple spot urine samples as a sampling strategy for the assessment of occupational exposure for glyphosate were examined.

Methods: A minimum of three spot urine samples were collected per work task; before the work task began; after the work task completion and the following first morning void. All samples were analysed separately for glyphosate using liquid chromatography tandem mass spectrometry and for creatinine. Differences in urinary glyphosate concentrations between the pre-task samples versus the post-task and the peak urinary samples were both analysed using paired Student t-tests. Determinants of exposure on glyphosate urine concentrations were evaluated using Pearson's correlation coefficients and linear regression. A multivariate mixed effect model were elaborated to compare the glyphosate concentrations between post-task and following first morning void samples. In these models, worker identity was entered as a random effect to account for the presence of correlations between repeated measurements from the same individuals.

Results: Peak urine glyphosate concentrations measured for work tasks were 2.5, 1.9, 1.9 and 7.4 μg L^{-1} (arithmetic mean, geometric mean, median and maximum value, respectively). Concentrations were highest in samples taken up to 3 hours after completing the work task. Regression analysis showed that workers who sprayed the day before the sampling task had

higher glyphosate concentrations in pre-task samples than those who did not spray the day before ($p < 0.01$). Similarly, workers who took breaks during the work task had higher peak urinary glyphosate concentrations ($p < 0.01$). The multivariate mixed effect model showed that the following first morning void samples were approximately a factor 0.7 lower than post-task values.

Conclusion: Occupational exposures to glyphosate among amenity horticulturalists are greater than those reported in environmental studies and comparable with previously reported agricultural studies. A suitable sampling strategy for occupational exposures to glyphosate is the collection of a spot urine sample up to 3 hours after completing the application of a glyphosate based pesticide product, which provides a reliable marker of peak exposure.

Keywords:

Biomonitoring
Pesticides
Glyphosate
Occupational exposure
Urine
Sampling strategy

Chemical compounds studied in this article: Glyphosate (Pubmed CID: 3496).

Introduction

Glyphosate, a broad spectrum post emerging herbicide, has recently received international attention due to its 'Group 2A – probably carcinogenic to humans' classification by the International Agency for Research on Cancer (IARC, 2016). The IARC classification differs to that of the European Chemicals Agency (ECHA, 2017), the European Food Safety Authority (EFSA, 2017), the United States Environmental Protection Agency (US EPA, 2016a) and the Food and Agriculture Organization of the United Nations of the World Health Organization (JMPR, 2016). EFSA have established an Acceptable Daily Intake (ADI) value of 0.5 milligram/kilogram of body weight per day (mg/kg bw/day) and an Acceptable Operator Exposure Level (AOEL) of 0.1 mg/kg bw/day for glyphosate (EFSA, 2015). Despite the widespread use of glyphosate, few publically available studies have investigated potential exposures during common occupational uses. There is also an increasing concern with respect to chronic low dose exposure of glyphosate based pesticides and adverse renal (Myers et al., 2016) and hepatic (Mesnage et al., 2017) health effects, with a necessity for studies to investigate this relationship further (Mills et al., 2017).

Biomonitoring is considered a reliable exposure assessment tool for pesticides once pharmacokinetics data is available. Biomonitoring involves the measurement of the pesticide or relevant biomarkers in biological samples such as blood or urine (Acquavella et al., 2003; Chester, 2010; Sexton et al., 2004).

Recently glyphosate has been added to national biomonitoring programmes in Canada (Haines et al., 2017), Germany (Conrad et al., 2017) and the United States of America (USA) (NHANES, 2018). It is generally accepted that more exposure data is required to characterise the range of exposures and to better distinguish exposure variations between individuals and different regions (Hoppe, 2013).

Biomonitoring data for glyphosate has been published for occupational exposures in the agriculture and horticulture sectors (Acquavella et al., 2004; Connolly et al., 2017; Curwin et al., 2007; Johnson et al., 2005; Mesnage et al., 2012). In addition, data on environmental exposures in Germany (Conrad et al., 2017; Hoppe, 2013; Krüger et al., 2014; Markard, 2014), the USA (Mills et al., 2017), Sri Lanka (Jayasumana et al., 2015) and in Ireland (Connolly et al., 2018) has also been obtained. Available data suggests that both occupational and environmental exposures do not exceed EFSA's ADI or the AOEL (Niemann et al., 2015).

It is often difficult to draw comparisons between the earlier published exposure studies for glyphosate due to the use of different analytical methods and/or sampling strategies. There is also some uncertainty regarding the half-life of glyphosate in humans (Faniband et al., 2017; IARC, 2016; Williams et al., 2000), which creates ambiguity regarding the sampling strategy that is most appropriate for occupational exposure assessment. Some occupational biomonitoring studies have analysed pooled urine samples collected over a 24 hour period, providing an estimate of average exposure over this sampling period (Acquavella et al., 2004; Lavy et al., 1992; Mesnage et al., 2012). However, this sampling strategy does not allow identification of peak exposures. Others have analysed a spot urine sample as a marker of the 24 hour exposure which could result in an under- or over-estimation of the actual exposure concentration (Connolly et al., 2017; Curwin et al., 2007; Scher et al., 2006). A spot sampling strategy may be considered more reliable for environmental exposure studies of the population, where recent occupational or personal use exposure is unlikely (Hinwood et al., 2002). For occupational exposure or regulatory risk assessments, where an estimate of the magnitude of exposure is required: collection and analysis of 24 hour urine samples, or multiple spot samples collected over a 24 to 48 hour period should provide a more reliable measure of the true exposure and variability (Kissel et al., 2005). However, this is not always practical or feasible and therefore spot sampling strategies are also needed.

To the authors' knowledge there are just two published studies reporting occupational exposure to glyphosate among amenity horticultural workers. The first study involved the collection of dermal and inhalation samples (Johnson et al., 2005) and the second (published by the authors of the current study) was a biomonitoring study (Connolly et al., 2017). Amenity horticulturists in the UK applied approximately 350,000 kg of glyphosate in a year (2012) (FERA, 2017), whereas the Irish amenity horticulture sector has a production value of over €70 million and employs over 1,300 workers (Horticulture Industry Forum, 2017). Connolly et al. (2017) assessed glyphosate exposures by collecting spot urine samples pre- and post- work tasks. Glyphosate exposure concentrations (geometric mean (GM) ((geometric standard deviation) (GSD)) 0.7 (1.1) µg L^{-1}) were detected. Considering the sampling strategy employed and the levels of detectable glyphosate concentrations found in the spot urine samples, the importance of this sector, its reliance on glyphosate and the numbers of horticultural workers that could potentially be exposed to glyphosate, the collection of more exposure data is warranted.

This current study describes the follow-up study, which aimed to characterise exposures within the same occupational group. The study provides a comprehensive assessment of occupational

exposures among amenity horticultural workers and examines the impact of work practices on exposure levels. The collection and analysing of multiple spot urine samples collected before, throughout the day and after pesticide applications allowed for an assessment of the suitability of the sampling strategy for occupational exposure assessment.

Materials and methods

Site description and study population

The measurement campaign took place during September 2016 to September 2017. Details regarding the study sites, which were managed by the Irish Commissioners for Office of Public Works (OPW) and the worker recruitment strategy have previously been published (Connolly et al., 2017). Briefly, three similar exposure groups (SEGs) were defined using information about the spraying methods used to apply glyphosate based pesticide products (Table 1; Figure 1). Recruitment was completed in coordination with the OPW Health and Safety Unit. The lead researcher (and lead author of the manuscript) delivered presentations outlining the project background and objectives and circulated project information leaflets to potential study participants. Participation in the study was voluntary. Workers gave informed consent and then completed a self-administered questionnaire, it provided relevant personal and work related details, as well as information on their use of pesticides outside of work and dietary habits.

Biomonitoring protocols were developed based on established protocols (Connolly et al., 2017; Galea et al., 2011; Health and Safety Authority, 2011; Health and Safety Executive (HSE), 1997). Project ethical approval was received from the National University of Ireland, Galway Research Ethics Committee (Ref: 16-July-19).

Biological monitoring

Urine collection

Biomonitoring exposure assessments were completed only on the days that workers used glyphosate based pesticide products. The researcher was on site observing all work tasks, and collected contextual information such as SEG, personal protective equipment (PPE) worn, climatic conditions, and any activities or work duties performed between samples and the duration of these activities.

Individual full urinary void spot samples were collected over the exposure assessment period using 1 and 2 litre containers, which were kept and analysed separately. The sample containers were pre-labelled and participants were asked to write the time and date for each void on the

container and to store samples in a cooler box until collection. The urine samples were collected by the researcher the morning following each task. Workers that participated in the study were asked to provide a minimum of three spot urine samples: i) before the work task began (pre-task sample), ii) within one hour of task completion (post-task sample) and iii) on the following morning (following first morning void). Participants were also given the option to provide urine samples for all additional voids over the exposure assessment period (pre-task to the following first morning void sample). Participants provided more than the minimum required three spot samples for 59% (17) of the tasks sampled (ranged between 3 and 7 samples per task). Of the spot urine samples given, a peak urinary sample was identified for each task, defined as the highest urinary glyphosate concentration measured during the sampling period.

To prevent sample contamination, written instructions on how to provide a urine sample were given to the workers including hygienic measures prior to sample collection. Following sample collection, the researcher measured and recorded the volume of each urinary spot sample using a volumetric cylinder. A 20 ml aliquot of the agitated spot sample was then transferred into a 20 ml Sterilin^{TM} pot and labelled with a unique identifier number, date and time. All samples were stored at -18°C within 24 hours and until laboratory analysis. The researcher used disposable nitrile gloves when handling all samples and all equipment and work surfaces were cleaned with a biological disinfectant before and after the handling. The measuring volumetric cylinders were tripled rinsed with distilled water between samples.

For the purposes of this study, the following time definitions were used:

Sampling time - time period between the pre and post-task samples;
Task time - time interval to complete the pesticide application task
Exposure assessment period - time interval between collecting the pre-task sample and the following first morning void sample.
Peak urinary concentration - the highest urinary glyphosate concentration detected after the pesticide application task, for the participant, over the exposure assessment period.

Urine sample chemical analysis

Chemical analysis was completed by the Health and Safety Laboratory (HSL), Buxton, UK. All samples were prepared and analysed for glyphosate following analytical methods previously described in Connolly et al. (2017) with minor alterations described here. In brief, glyphosate was extracted from urine samples (200 µl diluted with 800 µl deionised water) using strong anion exchange solid phase extraction (SPE) eluting into 10% formic acid in methanol.

The eluent was evaporated under a stream of nitrogen and reconstituted in 100 µl of 0.1% formic acid. Quantitative analysis was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Chromatographic separation was achieved on a Zorbax XDB-C8, 150 x 4.6mm, 5 µm (Agilent, Stockport, UK) column with mobile phases of 0.1% formic acid and acetonitrile with a gradient elution. The method was linear over the range 0- 20 µg L^{-1} and the limit of quantification (LOQ) was 0.5 µg L^{-1}. Creatinine analysis was also completed on all urine samples using an automated alkaline picrate method using a Pentra C400 clinical analyzer (Cocker et al., 2011).

Statistical analysis methods

Prior to analysis, all glyphosate concentration levels below the LOQ were imputed, using in SAS v 9.4 (SAS Institute, North Carolina, USA). A single random imputation method based on Maximum Likelihood Estimation was used (Lubin et al., 2004) as suggested for data of similar characteristics (Hewett and Ganser, 2007). The remainder of the statistical analysis was performed using Stata Software (StataCorp., 2015).

Urine data was analysed in micrograms per litre and creatinine adjusted values (µg L^{-1} and µmol/mol creatinine). Statistical analysis of creatinine adjusted samples excluded samples that had a creatinine level below 2 mmol L^{-1} or above 30 mmol L^{-1} (Cocker et al., 2011; EWDTS, 2002).

Visual inspection using graphical techniques suggested the biomonitoring data to approximate a log normal distribution and therefore the data was naturally log transformed for the analysis. All analysis was applied with biomonitoring data on the log-scale. Summary and descriptive statistics were calculated on the demographic and exposure variables. Pearson correlation coefficients were used to examine the correspondence between the log transformed post-task samples and the following first morning void samples. Differences between the average measured concentrations of the pre-task samples versus the post-task samples and the peak urinary samples were both explored using paired Student t-tests. One way Analysis of Variance (ANOVA) was used to examine concentration differences between the SEGs and the variance explained (r^2) explained by taking the sum of squares between groups and dividing it by the sum of squares total. Whereas univariate effects of certain factors such as spraying the day before or starting the task before sampling, on pre-task samples were examined using linear regression analysis.

The collection of post-task and following first morning void samples are the most commonly used and efficient sampling methods applied within human exposure studies. To advise on the most appropriate sampling method to apply after occupational exposure to glyphosate, the mean glyphosate concentration in the post-task sample and the following first morning void were compared in separate multivariate regression analysis. To account for the presence of correlations between repeated measurements from the same individuals, linear mixed effect regression models were applied with participant's identities included as the random effect. A restricted maximum likelihood (REML) fit was used. Optimal models were built using a forward selection approach with fixed effects (determinants of exposure) being selected by the strength of univariate associations ($p < 0.05$) and then by strength of association. Fixed effects considered in the modelling process included the similar exposure groups (pressurised lance, controlled droplet applicator and manual knapsack), the sampling time (minutes) and the type of sample (post-task sample vs following first morning void). Additional variables that were statistically significant (the quantity sprayed and whether the worker took a break) were excluded from the model due to high correlations with the fixed effect variables.

Results

Descriptive and summary statistics of demographic and task characteristics

Twenty (18 males and 2 females) amenity horticultural workers who apply pesticides as part of their work duties consented to participate in the study. Urine samples were collected for 29 work tasks involving glyphosate based pesticides. Demographic characteristics and details regarding the work tasks performed by study participants are presented in Table 2.

The concentration of glyphosate in 14 (48%) of the pre-task samples could have been influenced by work tasks performed in the days prior to this study and by starting the work task before giving the pre-task sample. For 6 (21%) of the pre-task samples, workers reported performing work tasks involving handling of glyphosate based products on the previous day to the measurement period. Similarly, for 10 (35%) of the pre-task samples (including 2 who were also involved with spraying the previous day), workers reported collecting, checking or handling potentially contaminated spraying equipment or other work equipment prior to providing the first sample (pre-task sample).

A large proportion of workers reported using pesticides outside of their job (90%), corresponding to 27 (93%) of the 29 tasks included in this study. However, none reported using

glyphosate based pesticides at home on the days before the sampling. The majority of the workers (60%) reported using pesticides at work for 6 - 7 months per year.

Levels of urinary pesticide concentrations

In this study, 125 spot urinary samples were collected and analysed for 29 work tasks. Participants provided between 3 and 7 individual spot samples per exposure assessment period. Participants that gave multiple (>3) spot urine samples over the exposure assessment period (n=17) allowed for a more accurate estimation of the peak urinary measurement (Figure 2) and to view glyphosate concentrations over time. There was no statistical significant difference in the average log transformed peak urinary concentrations where only the minimum 3 samples were provided versus those tasks where multiple samples were provided (Student t-test; p=0.14). For 13 (45%) of the 29 tasks collected, the peak concentrations were identified within the samples that were collected in addition to the minimum required (pre-task, post-task and following first morning void) samples. Another 31% of the peak samples were identified in post-task samples, whereas 24% comprised of following first morning void samples.

An overview of the glyphosate urinary concentrations (µg L^{-1}) for pre-task, post-task, following first morning void and peak concentration samples is provided in Table 3. The same summary statistics for the adjusted for creatinine concentrations values (expressed in µmol/mol creatinine) are provided in Table 4, to allow comparisons with the results from other glyphosate exposure assessment studies

Glyphosate concentrations were less than the LOQ in 34 (27%) urinary samples, of which 11 (38%) were pre-task samples and a further 11 (38%) were following first morning void samples (Table 5). For only two (7%) of the 29 work tasks all samples had non-detectable glyphosate concentrations, both belonging to the manual knapsack SEG.

Analysis of individual spot samples

Glyphosate concentrations (µg L^{-1}) in the post-task and following first morning void samples correlated moderately and similar results were found for creatinine adjusted values for the post-task sample and the following first morning void samples ($r = 0.64$, $p < 0.05$ for both).

The overall correlations between the two measurement metrics, creatinine adjusted and non-adjusted concentrations, were strong for pre-task, post-task and following first morning void samples ($r = 0.82$ - 0.89; $p < 0.001$) and moderate for peak samples ($r = 0.61$; $p < 0.001$).

Analysis of samples categorised by the time interval from task completion (e.g. post-task sample (within one hour of task completion), within three hours, 3-6 hours etc.) to the following first morning void were undertaken. This showed the highest median urinary glyphosate concentration in the sample collected following the post-task sample (up to 3 hours from task completion), (μg L^{-1} and μmol/mol creatinine (Figure 3(a) & (b))).

Evaluation of SEGs and potential determinants of exposure

Glyphosate concentrations in pre-task samples were statistically significantly different than the concentrations of both the post and peak samples (paired student t-test, $p < 0.0001$; μg L^{-1} and μmol/mol creatinine). No difference was observed between the pre-task concentrations and the concentrations of the following first morning void samples ($p = 0.4$).

When pre-task urine samples concentrations were deducted from peak urine sample concentrations, to account for possible environmental exposures, the minimum values among all exposure groups were negative (-0.10 to -2.06 μg L^{-1}) (Supplementary Information Table). This implies that pre-task sample concentrations were, in instances, higher than the peak sample concentrations (14% of tasks). Regression analysis suggested a scale factor of 4 higher pre-task sample concentrations ($p < 0.01$; $R^2 = 0.36$) for workers who sprayed the day before (n=6) compared to those who did not. The majority of these workers (67%) completed the pesticide application task with a handheld pressurised lance.

The pressurised lance SEG had the highest median and maximum values (Figure 4a) among the SEGs assessed, although the controlled droplet applicator SEG was slightly higher when adjusted for creatinine (Figure 4b). For post exposures, statistically significant differences between the SEGs glyphosate concentrations were observed for creatinine adjusted values (ANOVA; $p < 0.05$). Peak and following first morning void urinary concentrations differences across SEGs were borderline significant (ANOVA; $p = 0.059$) for non-adjusted creatinine values (μg L^{-1}), but were not different when adjusted for creatinine. Variance explained (r^2) by SEGs in the models ranged from 20 – 25%.

Workers who took breaks during the work task had a factor 2.1 higher peak urinary glyphosate concentrations ($\beta = 0.75$; $p < 0.01$) compared to those who completed the task without breaks. A similar pattern was observed for post-task samples concerning both adjusted and non-adjusted for creatinine concentrations.

The final elaborated multivariate mixed effect model comprised of type of spot sample (post-task vs following first morning void); the sampling time explained up to 18% of the variance in the urinary glyphosate concentrations (μg L^{-1}). The first morning voids were approximately a factor 0.7 lower than post-task values (β= -0.38; p=0.06) with similar results being observed when using creatinine adjusted values. For creatinine adjusted values the final model included task time and similar exposure group as fixed effects and explained 18% of the total variability in exposure (Table 6). Differences between the models could be attributed to biological differences, diuresis or creatinine fluctuations due to a number of reasons (e.g. age, sex, physical exercise) (Barr et al., 2005; Boeniger et al., 1993; Weihrauch et al., 2012) or as a result of the statistical analysis methodology or statistical power.

Discussion

Levels of urinary pesticide concentrations

This study provides new information on urine glyphosate concentrations and potential determinants of exposure among amenity horticulturists working with glyphosate based pesticide products. This study also reviewed the sampling strategy adopted, to identify the most suitable spot sample for biomonitoring when investigating occupational glyphosate exposures. The study results show that the mean urine glyphosate concentration increases because of the pesticide application task, suggesting occupational exposure to the amenity horticultural workers.

The peak glyphosate urine concentrations reported in this study had AM, GM, median and maximum levels of 2.5, 1.9, 1.9 and 7.4 μg L^{-1} respectively, which are higher than concentrations found in environmental studies. Hoppe (2013) reported mean urine glyphosate concentrations of 0.2 μg L^{-1} with a maximum value of 1.8 μg L^{-1} in their analysis of 182 urine samples (LOD 0.1 μg L^{-1}) across 18 European countries (Ireland was not included). Conrad et al. (2017) reported median and maximum urine glyphosate concentrations of 0.2 and 2.8 μg L^{-1}, respectively, from the analysis of 399 urine samples collected from German adults (LOD 0.1 μg L^{-1}).

Urinary glyphosate concentrations reported in the current study are comparable to exposure values reported for agricultural uses. A farm family glyphosate exposure assessment study (based in South Carolina and Minnesota) found that 60% of farmers urine samples analysed had detectable levels of glyphosate (LOD 1 μg L^{-1}), with highest exposures recorded on the

day of application (GM (GSD) of 3.2(6.4) µg L^{-1}) (Acquavella et al., 2004). Curwin et al. (2007) reported similar glyphosate concentrations in a study evaluating take home pesticide residues, which compared the urinary glyphosate concentrations of farm families to non-farm families. In this study glyphosate levels were actually higher for non-farm children, mostly likely due to residential use (greatest GM 2.7 µg L^{-1}; LOD 0.9 µg L^{-1}). A more recent study of glyphosate exposure among agricultural workers in Costa Rica found 70% of the 95 urine samples analysed to have detectable glyphosate concentrations (limit of quantification (LOQ) 0.1 µg L^{-1}) and reported a median and 95th percentile values of 0.4 and 1.8 µg L^{-1} respectively (Faniband et al., 2017).

Evaluation of SEGs and potential determinants of exposure

Glyphosate concentrations in the post-task samples, when adjusted for the concentration in the pre-task sample (post minus pre sample concentrations), in the current study are, on average, the same as those reported in our previous study (AM 0.6 µg L^{-1}) (Connolly et al., 2017). There was some variance within the SEGs between our two studies: the manual knapsack, the pressurised lance and controlled droplet applicator were 0.4 vs. 0.3 µg L^{-1}, 1.2 vs. 0.4 µg L^{-1} and 0.4 vs. 1.6 µg L^{-1}, respectively. Connolly et al. (2017) reported a higher maximum glyphosate concentration (10.7 µg L^{-1} vs. 7.4 µg L^{-1}) than found in the current study. The highest published urine glyphosate concentration reported in the literature (233 µg L^{-1}) was in an agricultural exposure assessment study for farmers in South Carolina (Acquavella et al., 2004). This would correspond approximately to 8.3% of the EFSA's AOEL (0.1 mg/kg bw/day) (Niemann et al., 2015) and is a magnitude higher than exposure concentrations reported in the current study.

When glyphosate exposure (AM) concentrations were examined using the peak minus the pre-task concentrations (shown in Supplementary Information Table), an even higher difference were seen for each of the SEGs compared to Connolly et al. (2017). Higher exposure concentrations was seen in tasks involving manual knapsack and pressurised lance applicators in our current study compared to Connolly et al. (2017) (1.0 vs. 0.3 µg L^{-1}; 2.5 vs. 0.4 µg L^{-1}, respectively); potentially, this was due to collecting additional urine samples after exposure. Conversely, exposure levels for the controlled droplet applicator group are lower in this study (0.8 vs. 1.6 µg L^{-1}). Prior to the study conducted by Connolly et al. (2017), the controlled droplet applicator was considered a low risk pesticide application method among the workers due to the use of a pre-mixed solution and the large droplet spray reducing potential spray drift.

The previous study found the highest median and maximum values in the controlled droplet applicator SEG. These results were communicated to the company and workers, which resulted in work practice improvements being implemented. These improvements, such improved PPE compliance, were very apparent during the on-site observations of the controlled droplet application SEG. Previously among Danish farmers it was shown that simple feedback of exposure measurement results to workers and their employers can lower exposure concentrations between 20% and 30% (Basinas et al., 2016).

Some workers were engaged in other pesticide related activities prior to sampling and thereby pre-task samples concentrations may have been inflated and not solely represent environmental exposures. Similarly, the following first morning void samples had a higher number of non-detects (38% tasks) than other spot samples, but some of the detectable samples had higher glyphosate concentrations than the previous samples. This could be due to workers (who provided samples on two consecutive days) starting the second day work task before providing the following first morning void sample (Figure 2). However, these findings could also reflect dermal absorption which is known to delay in uptake and therefore excretion (Garfitt et al., 2002).

Up to 25% of the variance in the peak glyphosate concentrations were explained by the SEGs which had significantly different average levels of exposure. This was not observed in the post-task concentration data likely due to the different sampling time patterns between SEGs. Higher urinary glyphosate concentrations, by at least a factor of two, were observed among workers who took breaks including lunch breaks and travel time between sites. These activities both involved donning and doffing PPE, which could cause exposure through transfer of residues. Increased sampling times were also associated with higher concentrations of urinary glyphosate, one third of the urinary glyphosate concentrations can be predicted by sampling time. The researcher observed good PPE compliance among the workers but possible opportunities for contamination were observed during donning and doffing PPE or when using mobile phones, adjusting facemasks or when adjusting nozzles on the applicator lances. From these observations, the potential for dermal and inadvertent ingestion exposures was identified and highlighted a need for investigation. Study results were used to develop a best practice guidance document for end users of plant protection products (Connolly, 2017).

Analysis of individual spot samples

Previous biomonitoring studies have used a range of sampling strategies such as collecting: single spot samples; multiple samples over 24-hour period; 24-hour composite samples, and/or collecting partial or full void samples. Studies have investigated the variability of differing sampling strategies (primarily in the context of environmental exposures) and suggested that partial void (spot) samples may over/under estimate exposures (Barr et al., 2005; Bradman et al., 2013; Kissel et al., 2005). One study investigating occupational exposures suggested collecting 24-hour samples as the biomonitoring 'gold standard' but acknowledged that it places a high burden on the study participants, which can result in compliance issues and research fatigue (Scher et al., 2006).

Biomonitoring studies also reported results using different metrics such as micrograms per litre (µg L^{-1}), full urinary voids adjusted for the volume of the void (µg L^{-1} adjusted for void volume) and creatinine adjusted spot samples (µmol/mol creatinine). Barr et al. (2005) recommended adjusting for creatinine in an environmental study. In the current study, urinary concentration data were expressed as micrograms per litre and micromoles per mole creatinine (µg L^{-1} and µmol/mol creatinine) and we found strong associations between these measuring metrics.

In this study, post-task and the following first morning void samples were evaluated for their ability to reflect peak exposure concentrations. There were strong positive correlations between the post-task sample and the following first morning urinary samples concentrations. Regardless of when the sample is collected, exposure information provided from the collection of spot urine samples after exposure, provides valuable information to regulators. For example, when calculating 'unit exposure' values for scenarios involving small quantity users (US EPA, 2016b) or when completing semi-quantitative risk assessment based on exposure intensity ranking. Results from this study suggest that, for tasks involving glyphosate based pesticide products, the sample collected post-task would be preferable than the following first morning void samples. The following first morning void samples may under estimate exposure as they were a factor 0.7 lower than post-task concentrations and 38% of them were non-detects in this study.

This study also reviewed the timing of collecting individual spot samples to assess glyphosate exposure to help identify the most appropriate spot sample to collect (if such a sampling strategy is necessary). The highest median levels are found in the sample sets collected following the post-task sample (up to 3 hours after task completion) for both µg L^{-1} and µmol/mol creatinine (Figure 3), which also was the only sample set (n=17) with all detectable

levels of glyphosate (Table 5). Ideally, obtaining two urine samples following a work task, involving glyphosate based pesticides, would allow the estimation of the peak concentration values. If only one sample is to be collected, then the sampling strategy should have a lag period (of 1-2 hrs) from finishing the work task before the collection of post exposure samples. Spot sampling may be preferable for exposure studies as it reduces the number of samples required. Our study has shown that spot samples may over/under-estimate the concentration but can be a good predictor of exposure and in many circumstances may be the only practical approach. The optimum frequency and timing of collecting samples is essential when developing a sampling strategy for obtaining biomonitoring samples (Barr et al., 2006), especially for occupational exposures, to ensure the accuracy of risk assessments.

Issues to consider

More than 25% of our collected samples were below the limited of detection. When present, the method of choice for handling such values (i.e. non-detects) may impact on the results and conclusions drawn by a study (Helsel, 2010; Hewett and Ganser, 2007). This is an issue that needs be considered when results from different studies with left-censored date are compared between them. In the present study levels of non-detects were imputed using a single random imputation method based on a Maximum Likelihood Estimation (MLE) method. Previous research has shown that for datasets with similar sizes and proportions of non-detects to ours the MLE method produces results for means and 95th percentiles that are less biased and more precise compared to other estimation methods - e.g. substitution, log-probit regression (Hewett and Ganser, 2007) - and is considered the 'best overall estimator'(Gibbons et al., 2001). The most commonly used methods of simple substitution (e.g. by assigning a value equal to one-half the detection limit) are advised only for certain scenarios depending upon the underlying GSD and a small proportion (<10%) of censored values (Hewett and Ganser, 2007; Lubin et al., 2004).

In the present study samples were not analysed for Aminomethylphosphonic acid (AMPA). AMPA, a biodegradation product of glyphosate, has been suggested to share a comparable toxicity profile and to contribute in exposure within environmental studies (Conrad et al., 2017; Silva et al., 2018). However, in acute ingestion studies, AMPA concentrations were found in orders of magnitude lower than glyphosate concentrations (Hori et al., 2003; Zouaoui et al., 2013). As it is not a significant human metabolite (IARC, 2016), it was not included in our analysis.

The current study does have some limitations. A small sample size prevented us from elaborating extensive multivariate exposure determinant models and which might have impacted on the robustness of the reported size effects in the elaborated regression models. The imbalance of the sample population in relation to gender and sex related differences may be another limitation impacting the representativeness of our results regarding the exposures of female workers. Differences for creatinine adjusted values between genders have been reported in an environmental glyphosate exposure study (Conrad et al., 2017). However, our field observations and liaising with industry stakeholders indicate that, in Europe, it is mostly males conducting the tasks of concern and therefore we consider our results as representative of the industry in question.

Conclusions

Our study provides information on occupational exposures to glyphosate among amenity horticulturalists. Exposure data, such as that presented in this study, is required to help evaluate the potential adverse human health effects from chronic low dose exposure to glyphosate and for the continued refinement of risk assessments for regulatory and exposure science purposes. Our study also suggest that the collection and analysis of urine samples given up to 3 hours after task completion can be a suitable sampling strategy for estimating potential occupational exposures to glyphosate.

Our findings suggest that amenity horticulturists, largely complying with workplace exposure controls, have low levels of glyphosate exposures. Workplace observations identified dermal and inadvertent ingestion as possible routes of exposure and future work is required to quantify the contributions of these routes to total glyphosate exposure.

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Tables

Table 1: Characteristics of established similar exposure group (SEG).

Similar Exposure Group	***Glyphosate products used (& glyphosate concentration of product)***		***Description of pesticide spray applicator***
	Product	***Conc. (g L^{-1})***	
Manual knapsack	Roundup Biactive XL Clinic Ace Roundup Biactive Pistol Roundup XL	360 360 360 250 360	A handheld lance connected to a knapsack, carried on the workers back. In this study, workers typically sprayed 10 – 15 litres of the pesticide solution, (approximately 1 part concentrate to 10 parts water). The applicator was used for spot spraying weeds, spraying footpaths and chemical edging around gardens (Figure 1a).
Pressurised hand held lance	Roundup Biactive XL Pistol Glyfos Rambo 360 Roundup Gold 450	360 250 360 360 450	A handheld lance (100 – 200 kPa) connected to a motorised portable applicator. Workers typically used 1 part concentrate to 10 parts water for total weed control in large open areas and courtyards (Figure 1b). One worker used a portable 5 litre SteelMaster V5 professional sprayer, a manual handheld lance (operated at 600 kPa), used to spray above head height to maintain the integrity of historic buildings.
Controlled droplet applicator	Nomix Dual Roundup XL	120 360	The controlled droplet applicator has a function where the user can control the droplet size, which reduces spray drift. This applicator is used with a premixed solution, for clearing footpaths and for total weed control (Figure 1c). Workers used on average, 1-3 litres of the pesticide product.

Table 2: Demographics, work and sampling characteristics. Data are presented as number of observations (percent) or mean values (range) for parameters on a continuous scale.

Variable		
Participant and work characteristics	**Number (N)**	**Percentage (%)**
Participants	20	100
Male	18	90
Female	2	10
Work tasks	29	100
Workers that sprayed pesticides the day before the task	6	21
Workers that started the work task before assessment	10	35
Participants that smoke	2	10
	Arithmetic Mean	**Range**
Age (years)	48	32-60
Work characteristics & Personal Protective Equipment (PPE) use	**Arithmetic Mean**	**Range**
Length of time working with pesticides (years)	22	10-35
Length of exposure assessment period (hrs)	22	17-26
Length of the task time in each SEG (minutes)		
Manual knapsack	116	37-360
Controlled droplet applicator	158	38-286
Pressurised applicator	226	60-380
Length of sampling time in each SEG (minutes)		
Manual knapsack	186	86-366
Controlled droplet applicator	205	65-420
Pressurised applicator	375	115-470*
Participants pesticide & PPE use	**Tasks (N)**	**Percentage (%)**
Glove use - Yes	29	100
Tyvek use - Yes	26	90
RPE use - Yes	28	97
Which tasks did the workers complete at least once?		
Collection of the pesticide from the store	24	83
Mixing and loading the chemical	19	66
Spraying the chemical	28	97
Cleaning the equipment	15	52
Did the worker take a break during the task?		
Yes	13	45
Did the worker reuse PPE?		
Reuse PPE	16	55
Do not reuse PPE	13	45
Uses pesticides outside of work	27	93
Similar exposure groups (SEGs)	**No. participants**	**No. tasks**
Manual knapsack	10	12
Controlled droplet applicator	5	7
Pressurised lance	6	10

*One measurement was excluded from the range as one participant forgot to give a post task sample.

Table 3: Biological monitoring results (µg L^{-1}) describing urinary glyphosate concentrations for pre-task, post-task, following morning voids and peak samples described by the mean, standard deviation, 10th percentile, median, 90th percentile values and the range. Results are presented as glyphosate and per similar exposure group.

Variable	k	n	<LOQ N (%)	AM	SD	GM (CI 95%) [a]	P10	P90	Min	Max
Combined glyphosate SEGs										
Pre sample	20	29	11 (38)	1.08	1.20	0.68 (0.47 - 0.98)	0.19	2.99	0.14	5.44
Post sample	20	28[#]	5 (18)	1.72	1.53	1.17 (0.82 - 1.68)	0.41	4.95	0.17	5.51
Morning void	20	29	11(38)	1.32	1.32	0.83 (0.56 – 1.22)	0.17	2.82	0.13	6.14
Peak sample	20	29	2 (7)	2.53	1.89	1.90 (1.40 - 2.57)*	0.68	5.51	0.39	7.36
Manual knapsack										
Pre sample	10	12	6(50)	0.91	1.46	0.50 (0.26 - 0.96)	0.17	0.91	0.14	5.44
Post sample	10	12	2(17)	1.30	1.10	0.93 (0.52 - 1.64)	0.47	3.28	0.17	3.38
Morning void	10	12	7(58)	0.86	0.89	0.55 (0.30 – 1.02)	0.17	2.59	0.16	2.62
Peak sample	10	12	2 (17)	1.89	1.42	1.44 (0.87 - 2.39)*	0.47	3.38	0.39	5.04
Pressurised lance										
Pre sample	6	10	4 (40)	1.15	1.01	0.80 (0.41 - 1.54)	0.26	2.81	0.23	2.99
Post sample	6	9[#]	0(0)	2.38	1.74	1.82 (0.99 - 3.36)	0.51	5.11	0.51	5.11
Morning void	6	10	1(10)	2.05	1.70	1.54 (0.87 – 2.75)	0.52	4.71	0.48	6.14
Peak sample	6	10	0(0)	3.65	2.12	3.06 (1.91 - 4.91)*	1.23	6.75	0.95	7.36
Controlled droplet applicator										
Pre sample	5	7	1(14)	1.26	1.08	0.92 (0.39 - 2.10)	0.20	3.38	0.21	3.38
Post sample	5	7	3(43)	1.61	1.82	1.01 (0.39 - 2.61)	0.30	5.51	0.31	5.51
Morning void	5	7	3(43)	1.05	0.95	0.68 (0.25 – 1.87)	0.13	2.82	0.13	2.82
Peak sample	5	7	0(0)	2.01	1.72	1.54 (0.76 - 3.10)*	0.68	5.51	0.68	5.51

*k is the number of participants in the group
n is the number of tasks in the group

[a] The geometric mean and 95% confidence interval (0.95 probability of containing the population mean) as GM(CI 95%).# One post-task sample was not given as the participant forgot to collect the void for analysis.
‡ These values are due to the pre sample having higher concentrations than the peak values, causing a minus values. In both cases, the worker had sprayed glyphosate based pesticide products the day before sampling.
AM = Arithmetic Mean, SD = Standard deviation, P10 = 10th percentile, P90 = 90th percentile, Min = Minimum, Max = Maximum.
* Log-transformed concentrations were statistically significant between SEGs ($p < 0.05$)

Table 4: Biomonitoring results (µmol/mol creatinine) describing urinary glyphosate concentrations for pre-task, post-task, following morning voids and peak levels for each work activity described by the mean, standard deviation, 10th percentile, median, 90th percentile values and the range. Results are presented as glyphosate and per similar exposure group.

Variable	k	n	< 2 mmol L^{-1} or >30 mmol L^{-1}	<LOQ N (%)	AM	SD	GM (CI 95%) [a]	P10	P90	Min	Max
Combined glyphosate SEGs											
Pre sample	18	26	3	11 (38)	0.76	0.74	0.47 (0.31 - 0.72)*	0.13	2.06	0.10	2.52
Post sample	18	25[#]	3	5 (18)	1.46	1.75	0.93 (0.63 - 1.40)*	0.30	3.10	0.09	8.65
Morning void	20	27	2	11(38)	0.98	0.94	0.60 (0.39 – 0.92)	0.16	2.74	0.05	3.52
Peak sample	20	29	0	2 (17)	2.84	3.25	1.69 (1.13 - 2.53)	0.41	8.65	0.16	12.72
Manual knapsack											
Pre sample	9	11	1	6(50)	0.42	0.56	0.27 (0.15 - 0.49)*	0.12	0.51	0.10	2.06
Post sample	10	12	0	2(17)	0.76	0.48	0.59 (0.34 - 1.01)*	0.27	1.39	0.09	1.49
Morning void	10	12	0	7(58)	0.56	0.49	0.36 (0.19 – 0.71)	0.09	1.31	0.05	1.46
Peak sample	10	12	0	2 (17)	3.14	4.55	1.34 (0.57 - 3.13)	0.34	12.22	0.16	12.72
Pressurised lance											

Pre sample	6	8	2	4 (40)	0.74	0.67	0.52 (0.24 - 1.12)*	0.14	2.04	0.14	2.04
Post sample	5	9	1	0(0)	2.01	2.59	1.24 (0.59 - 2.61)*	0.34	8.65	0.34	8.65
Morning void	5	9	1	1(10)	1.45	1.14	1.04 (0.51 – 2.13)	0.22	3.52	0.22	3.52
Peak sample	6	10	0	0(0)	2.54	2.33	1.88 (1.05 - 3.36)	0.61	6.03	0.46	8.65
Controlled droplet applicator											
Pre sample	5	7	0	1(14)	1.33	0.81	1.03 (0.49 - 2.34)*	0.18	2.52	0.18	2.52
Post sample	3	4	3	3(43)	2.34	1.42	1.99 (0.67 - 5.93)*	0.89	3.93	0.88	3.93
Morning void	4	6	1	3(43)	1.11	1.05	0.72 (0.24 – 2.18)	0.20	2.74	0.20	2.74
Peak sample	5	7	0	0(0)	2.75	1.65	2.17 (0.98 - 4.80)	0.41	5.30	0.41	5.30

*k is the number of participants in the group

n is the number of samples in the group

[a] The geometric mean and 95% confidence interval (0.95 probability of containing the population mean) as GM(CI 95%).<2 mmol L^{-1} >30 mmol L^{-1} is the number of samples that are were excluded from analysis, only samples within the creatinine levels above 2 mmol L^{-1} and below 30 mmol L^{-1} were included.

one post-task sample was not given as the participant forgot to collect the void for analysis.

The samples that are below the limit of detection (<LOQ) by Number (N) and by percentage (%)

AM = Arithmetic Mean, SD = Standard deviation, P10 = 10^{th} percentile, P90 = 90^{th} percentile, Min = Minimum, Max = Maximum.

* Log-transformed concentrations were statistically significant between SEGs ($p < 0.05$).

Table 5: Number of samples taken within the exposure assessment period.

Samples	**Pre – task samples**	**Post –task samples***	**3rd sample**	**4th sample**	**5th sample**	**6th sample**	**Following morning void**
N	29	28	17	15	6	2	29
< LOQ ($0.5\ \mu g\ L^{-1}$)	11 (38%)	5 (18%)	0 (0%)	8 (53%)	3 (50%)	0 (0%)	11 (38%)

N = Number of tasks

*The post task sample was provided within one hour of the task completion.

Table 6: Forward built mixed effects models results for the log transformed urinary concentrations of glyphosate, unadjusted and adjusted for creatinine (μg L^{-1} and μmol/mol creatinine, respectively), with participant's identification number as taken as a random effect. Regression analysis results describes the effects of the post-task samples and the following morning samples measured from 20 workers over 29 pesticide application tasks.

Covariate	μg L^{-1}			μmol/mol creatinine		
	β	Se	p	β	Se	p
Intercept	-0.34	0.28	0.21	-0.71	0.35	0.04
Type of Sample (1/0)						
Morning void	-0.38	0.21	0.06	-0.35	0.17	0.04
Post work task	Ref	-	-	Ref	-	-
Sampling time (minutes)	0.003	0.001	0.02	0.001	0.002	0.71
Similar exposure groups (1/0)						
Pressurised lance				1.10	0.37	0.00
Controlled applicator				0.80	0.47	0.09
Manual knapsack				Ref	-	-
Between variance (naïve model)*	0.20 (0.40)			0.57 (0.68)		
Within variance (naïve model)*	0.60 (0.57)			0.35 (0.44)		
Total variance explained	18%			18%		

β =regression coefficient for log transformed data; se=standard error; p=p-value.

* The naïve model is the results from the model without the inclusion of any fixed effects.

Figures

(a) (b) (c)

Figure 1: (a) Manual Knapsack sprayer with a handheld manual lever **(b) Pressurised lance** is a motorised portable applicator with a handheld lance (2-3 bar pressure) **(c) C*ontrolled droplet applicator*** with adjustable droplet size and pre-mixed solution.

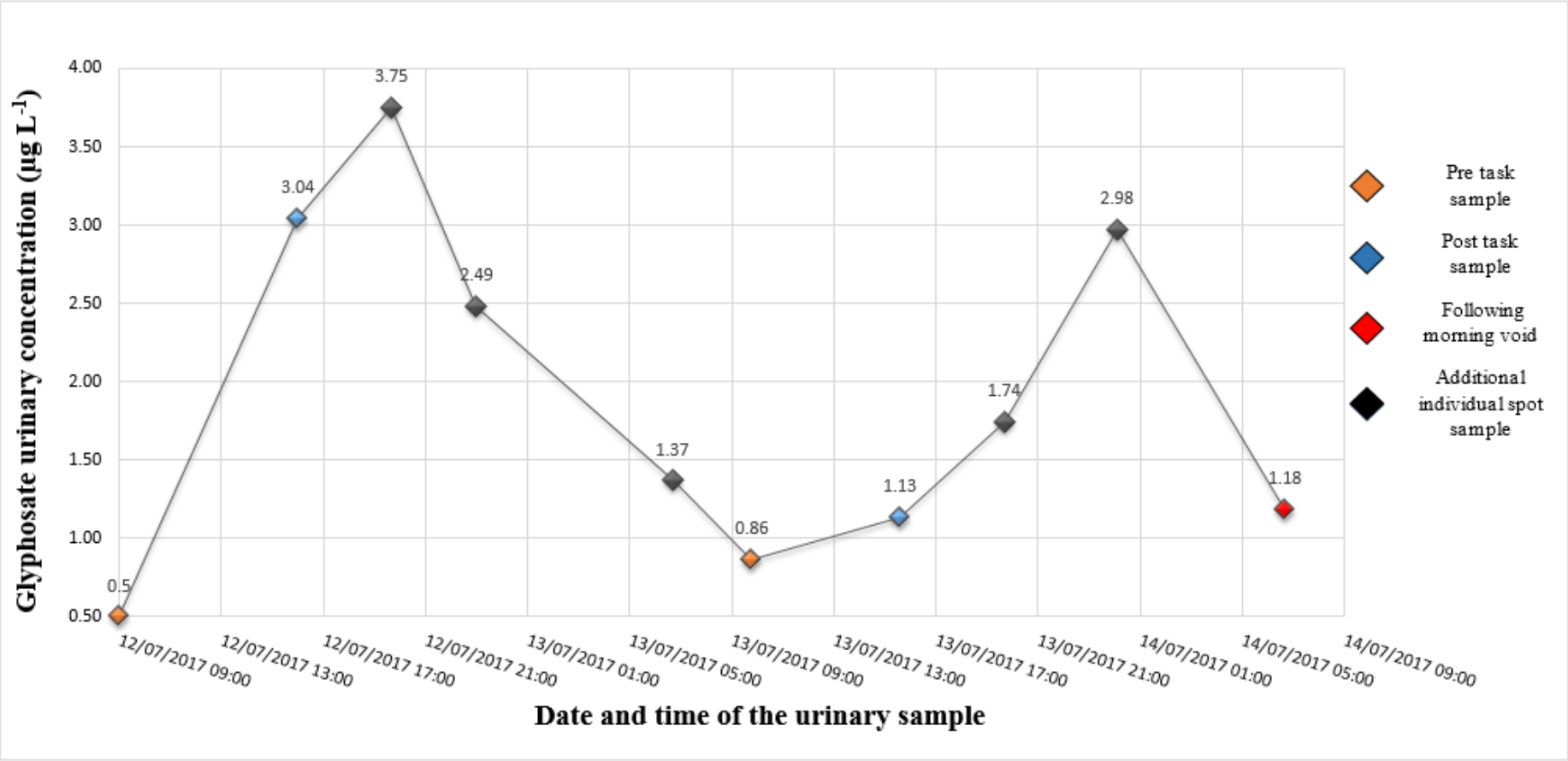

Figure 2: Urinary glyphosate concentrations of spot samples over 48 hour period using a pressurised lance. Data from a single worker who sprayed glyphosate over two consecutive days using a 6 bar pressurised knapsack with a handheld lance. On both days, the worker started work at 8am and finished at approximately 4:30 pm. Day 1, the worker sprayed approximately 14 litres, with only approximately 1 ½ hours spent on the pesticide application task, with 7 ½ hours on between work activities (e.g. driving, paperwork, breaks etc.). Day 2, the worker sprayed 19 litres, with approximately 3 ½ hours spent on the pesticide application task and 4 ½ hours on between work activities. *The first data point was a non-detect and the limit of detection has been substituted and axis amended to start at this value.

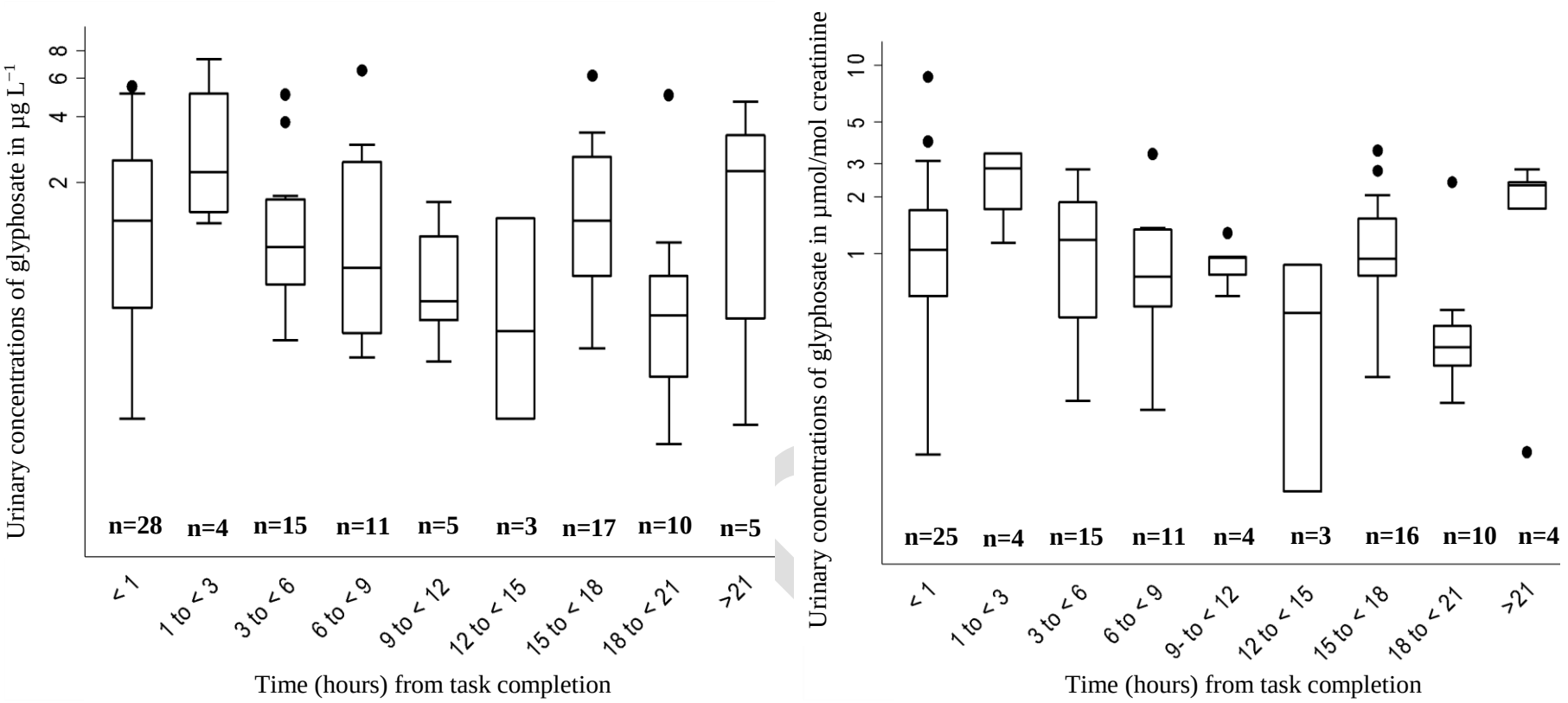

Figure 3: Urinary concentrations of glyphosate from post sample (within one hour of task completion) and then in time intervals after task completion **a)** in micrograms per litre (µg L^{-1}) and **(b)** in micromole per mole creatinine (µmol/mol)

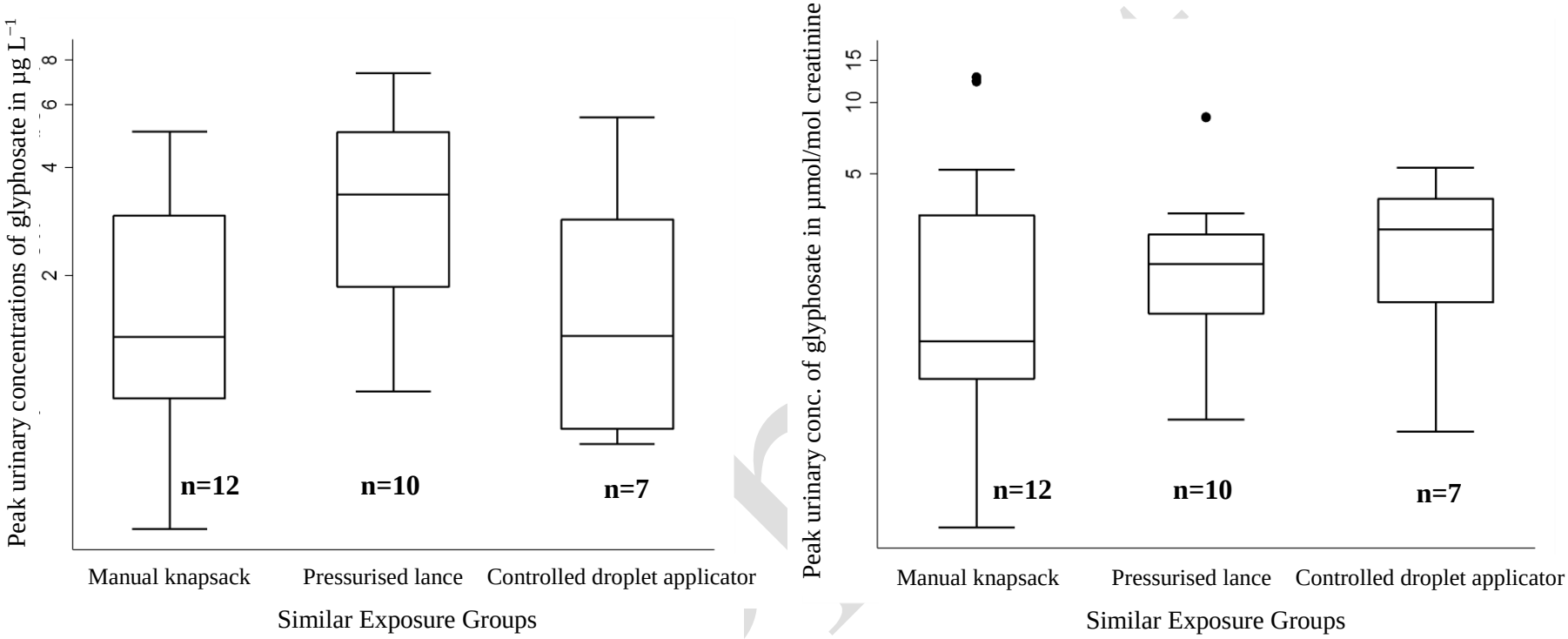

Figure 4: Boxplot showing the peak urinary concentrations of glyphosate **(a)** in micrograms per litre (µg L^{-1}) and **(b)** in micromole per mole creatinine (µmol/mol) in each SEG. The box is the 25th to the 75th percentile, the line within the box is the median and the whiskers the lower and the upper values.

Supplementary Information

Table 1: Biological monitoring results (µg L^{-1}) describing urinary glyphosate concentrations for adjusted values (peak minus pre-task samples) described by the mean, standard deviation, median, 10th percentile, 90th percentile values and the range. Results are presented as glyphosate and per similar exposure group.

Variable	k	n	<LOQ N (%)	AM	SD	Median	P10	P90	Min	Max
Combined glyphosate SEGs										
Adjusted (Peak – Pre samples)	20	29	11 (38)	1.49	1.88	0.91	-0.19‡	4.60	-2.06‡	5.31
Manual knapsack										
Adjusted (Peak – Pre samples)	10	12	6(50)	0.98	1.71	0.69	0.03	2.77	-2.06‡	4.75
Pressurised lance										
Adjusted (Peak – Pre samples)	6	10	4 (40)	2.50	1.71	2.26	0.23	4.84	-0.10‡	5.31
Controlled droplet applicator										
Adjusted (Peak – Pre samples)	5	7	1(14)	0.75	1.98	0.54	-2.03‡	4.60	-2.03‡	4.60

k is the number of participants in the group
n is the number of tasks in the group
‡ These values are due to the pre sample having higher concentrations than the peak values, causing a minus values. In both cases, the worker had sprayed glyphosate based pesticide products the day before sampling.
AM = Arithmetic Mean, SD = Standard deviation, P10 = 10th percentile, P90 = 90th percentile, Min = Minimum, Max = Maximum.